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Biological Effects of Indigenous Medicinal Plant (*Apium Graveolens* L)

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Abstract
The crude Hexane extract of *Apium graveolens* seeds were screened for their antifungal, antibacterial, insecticidal, antileishmanial and brine shrimp lethality activities. The extracts were tested against six different species of human pathogenic bacteria by using Agar well diffusion method and six fungal strains by agar tube dilution method. The Hexane extract exhibited strong inhibitory activity against animal pathogens *Trichphyton longifuss* (80%) and *Microsporum canis* (80%), this extract was found to be devoid of insecticidal, antileishmanial, contact toxicity, antibacterial and brine shrimp lethality activities.
Keywords: Hexane, Agar, Bioassays tests, Animal pathogen

INTRODUCTION

Celery (*Apium graveolens* L) belonging to the family (Umbelliferrie), an annual or biennial herb. It is in flower from June to August, and the seeds ripen from August to September. The flowers are hermaphrodite (have both male and female organs) and are pollinated by Flies [1]. And the plant is self-fertile. The plant prefers light (sandy), medium (loamy) and heavy (clay) soils. The plant prefers acid, neutral and basic (alkaline) soils and can grow in saline soil. And can be grow in semi-shade (light woodland). It requires moist soil. It prefers a rich moist soil in sun or semi-shade [2], but with some shade in the summer. It is tolerant of saline soils [3]. Wild celery has a long history of medicinal and food use. It is an aromatic bitter tonic herb that reduces blood pressure, relieves indigestion, stimulates the uterus and is anti-inflammatory [2]. The methanolic extract of Celery seeds was also investigated for bioactive compounds and resulted in the isolation and characterization of mosquitocidal, nematicidal, and some other antifungal compounds [4]. The ripened seeds, herbs and roots are apparent, carminative, diuretic, emmenagogue, galactogogue, nervine, stimulant and tonic [4-5]. Wild celery is said to be useful in cases of hysteria, promoting restfulness and sleep and diffusing through the system a mild sustaining influence [6]. This herb shouldn't be prescribed for pregnant women. An essential oil obtained from the defined plant has a calming effect on the central nervous system. Some of its constituents have antispasmodic, sedative and anticonvulsant actions. It has been shown to be of value in treating high blood pressure [6].

MATERIAL AND METHODS

Plant material Used

The plant is collected in August and September, from the Khyber Pakhtunkhwa, Pakistan, were identified by Medicinal Plant Botanist, Pakistan Council of Scientific and Research Institute Peshawar, Pakistan.

Extraction

Seeds of the subject plants were extracted with Hexane by placing them overnight and then concentrated with the help of rotary apparatus.

ANTIFUNGAL ACTIVITY

The Antifungal bioassay was resoluted by using Agar well dilution method [7]. Twenty-four mg of crude extract and 12 mg of pure compound was dissolved in 1mL sterile Dimethyl sulfoxide (DMSO) serving as stock solution. Sabouraud dextrose agar (SDA, Sigma-Aldrich, Germany), 40% glucose was prepared by mixing (SDA) 32.5 gm 500-1 ml distilled water. The contents were dissolved and dispensed as 4 ml volumes into screw capped tubes. Autoclaved at 121ºC for 15 minutes and then cooled to 50 ºC. The non-solidified SDA media was mixed with (66.6 µL) giving a final concentration of 400 and 200 ug ml-1 of the crude extract and pure compound respectively. Tubes were then allowed to solidify in slanting position at room temperature. Each tube was inoculated with 4 mm diameter piece of inoculums removed from a seven-day-old culture of fungus. For non-mycelial growth, an agar surface streak was employed. Other media supplemented with (DMSO) and reference antifungal drugs used as negative and positive control respectively. The tubes were incubated at 27-29 ºC for 7-10 days. A relative humidity was of 40-50% with an open pan of water in the incubation room. Cultures were examined twice weekly during incubation. Growth in the media was determined by measuring linear growth (mm) and growth inhibition calculated with reference to the negative control.

$$Inhibition\,(\%) = 100 - \frac{Growth\ in\ Sample\,(mm)}{Growth\ in\ Control\,(mm)} \times 100$$

ANTIBACTERIAL ACTIVITY

Test crude Hexane extract was screened against various human pathogens including *Staphylococcus aureus, Eschericha coli, Pseudomonas aeruginosa, Salmonella typhi, Bacillus subtilus, Shigella flexenari* by using Agar well diffusion method [8]. In these bioassay three types of media were prepared i.e. Solid medium (nutrient agar), Semi-solid medium (soft agar) and liquid medium (nutrient broth). Solid medium was prepared by taking 28 gm nutrient agar and 1L of distilled water. Medium was dissolved and autoclaved it at 121ºC for 15 min. cooled it up to 45 ºC and then 40-50 ml media in sterile 120 mm diameter Petri dish was poured. Median was allowed to solidify, and then kept it at room temperature to check the sterility of the prepared media. Semi-solid medium was prepared by taking 0.8g agar and 100ml of distilled water. Agar was dissolved and dispensed in approx 7mL soft agar in screw-capped test tubes. Autoclaved at 121 ºC for 15 minutes and stored in refrigerator. Liquid medium was prepared in such away that 0.8 gm of agar and 100ml of distilled water was taken and then dissolved the broth and dispensed approximately 3ml broth in screw capped test tubes. The materials were autoclaved at 121ºC for 15 min and stored in 4 ºC On the first day single colony of bacterial culture was inoculated in nutrient broth and incubated it at 37 ºC for 24 hrs. On the second day soft agar tube was taken, melted and cooled it up to 40 ºC then added 100µL of fresh bacterial culture shaked it well and then poured it on to nutrient agar containing plate. The plate was rotated so that even distribution of the culture was obtained; the lawn was allowed to solidify. And these Petri plates were marked well by using 6 mm-diameter sterile borers.

BRINE SHRIMP CYTOTOXICITY

Brine-shrimp (*Artemia salina*) eggs were used and the brine-shrimp cytotoxic activity of the subject material was found by adopting the standard method [9]. Three different concentrations 10, 100 and 1000µg/ml of test sample were prepared by dissolving in DMSO as the solvent. Seawater was prepared by dissolving commercially available ea salt (3.8g) into tap water (1:1). Shrimp was used 48-72 hrs after the initiation of hatching. Ten shrimps seawater (5ml) and different amounts of each test sample were put in a vial. Two other vials were supplemented with solvent and reference cytotoxic drug serving as negative and positive controls respectively. Etoposide (LD_{50} = 7.465 µg/ml) was used as the standard reference cytotoxic drug. All vials were incubated at 25-27 ºC for 24 hrs and the survived brine shrimps were counted. The data was analyzed with Finny computer program to determine LD_{50} values with 95% confidence interval.

INSECTICIDAL ACTIVITY

This test is used to assess the direct insecticidal actions of pure natural products or plant extracts. This method unambiguously demonstrates if a compound or extract is lethal to certain types of insects on contact and not because of volatility of samples [10]. Suitable quantities (1000, 500, 100, 50, 10 ppm) of test samples were dissolved in a volatile organic solvent and these solutions were then coated on the inner surface of 20 ml glass vials (two to five replicates for each concentration). Each glass vial was rotated by hand until the test solution was distributed on the vial inner wall and floor, and the solvent had mostly evaporated. Then each vial was placed in a fume hood for 10 minutes to ensure complete removal of the carrier solvent. Care should be taken to ensure that a uniform quantity of the test material is coated on the inner surface of the vial. When the solvent was completely evaporated, five test insects (or larvae) were placed carefully in each vial with sufficient food (i.e. the natural diet for that particular insect, such as leaves, grain, etc., or artificial diet which is different for different insects). The survival of the insects was assessed after 24-48 hours. Controls consist of test insects (or larvae) in vials, treated only with the carrier solvent. Survival of such controls should average over 95%. Data was analyzed with a Finney computer program (Probit analysis) to determine LC_{50} values and 95% confidence intervals.

ANTILEISHMANIAL ACTIVITY

Promastigotes are the extra cellular flagellated form of the parasite and they can be used for in vitro determination of leishmanicidic activity of natural compounds. Leishmanial promastigotes can be obtained from the infected animals or human in endemic areas (Mingarro, *et al* 2003). Leishmanial promastigotes were cultured in sterile 25 cm^2 tissue culture flask in tissue culture medium M-199 suppleemented with the help of improved Neubauer chamber under a microscope. Parasites were diluted with the fresh medium to a final concentration of 2.0 x 106 parasites mL-1. One mg of compound was dissolved in 50µl of absolute Me OH or DMSO (Dimethyl sulfoxide) and the volume was made up to 1.0 ml with the culture medium. In a 96 well micro titer plate, 90µl of

the parasite culture (2.0x 106 parasites mL^{-1}) was placed and 10µl containing various concentrations of the experimental compound was added in the culture. 10µl of PBS (phosphate buffered saline, pH 7.2 containing 0.5% DMSO) were added separately as positive control. The plates were incubated at 25 ºC in the dark for 3-5 days during which control organism multiplied 3-6 times. The culture was examined microscopically on an improved Neubauer chamber and ED_{50} value of compounds possessing antileishmanial activity was calculated.

RESULTS AND DISCUSSION

Recently, much attention has been directed toward extracts and biologically active compounds isolated from popular plant species. The use of medicinal plants plays a vital role in covering the basic health needs in developing countries, and these plants may offer a new source of antibacterial, antifungal and antiviral agents with significant activity against infective microorganisms [11-12]. Scientific analysis of plant components follows a logical pathway. Plants are collected either randomly or by following leads supplied by local healers in geographical areas where the plants are found. Initial screening of plants for possible antimicrobial activities typically begins by using crude aqueous or alcohol extraction and can be followed by various organic extraction methods. Since nearly all of the identified components from plants are active against microorganism are aromatic or saturated organic compounds, they are often obtained through initial ethanol or methanol extraction [13]. Antifungal activity of the medicinal plant was tested against *Trichphyton longifuss, Candida albicans, Aspergillus flavus, Microsporum canis, Fusarium solani* and *Candida glabrata*. Concentration of each sample was taken as 200 µg ml^{-1} of DMSO. Growth in the medium containing the extract was determined by measuring the linear growth in mm and % growth inhibition was calculated with reference to the negative control. The standard drug used was Micanazole 70 and Micanazole 98.4. The result indicated that the crude Hexane extract showed strong inhibitory activity against *Trichphyton longifuss* (80%) and *Microsporum canis* (80%). *Trichphyton longifuss* is responsible for a pathogenic characteristic, Cutaneous mycosis which causes a severe type of acute inflammatory infection of the hair and follicle called "Favus" [14]. It results in permanent hair loss and sometime infects the nails and skin. *Microsporum canis* is an animal pathogen responsible for cutaneous mycosis. It is the most common cause of ringworm infection of hair and skin in dogs and cats. Human infection usually acquired by contact with infected animals, particularly cats. Table 2 shows the antibacterial activity of the extract, which was tested against *Eschericha coli, Bacillus subtilis, Shigella flexenari, phylococcus aureu, Pseudomonas aeruginosa* and *Salmonella typhi*. Standard drug used was imipenum (100 µg ml^{-1}). It displayed non-significant activity with zone of inhibition of 10 mm against *Salmonella typhi*. The extract was also screened against antileishmenial; insecticidal, contact toxicity and brine shrimp lethality studies but the extract did not display any significant activity in these bioassays.

Table 1. In Vitro Antifungal Bioassay of *Apium graveolens* Linn

Name of Fungus	Linear Growth (mm)		% Inhibition	Std. Drug MIC
	Sample	Control		(µg mL^{-1})
Trichphyton longifusus	20	100	80	Miconazole
C. albicans	100	100	0	Miconazole
A.flavus	100	100	0	Amphotericin B
Microsporum canis	20	100	80	Miconazole
F. solani	100	100	0	Miconazole
Candida glabrata	100	100	0	Miconazole

Table 2. In Vitro Antibacterial Bioassay of Apium graveolens Linn

Name of Bacteria	Sample Inhibition Zone (mm)	Zone of inhibition of Std. Drug(mm)
Eschericha coli	_	25
Bacillus subtilis	_	26
Shigella flexenari	_	24
Staphylococcus aureu	_	17
Pheudomonas aeruginosa	_	17
Salmonella typhi	10	21

CONCLUSION

The research work revealed that the Hexane extract exhibited strong inhibitory activity against animal pathogens *Trichphyton longifuss* (80%) and *Microsporum canis* (80%). The volatile oil showed antifungal activities and is active against many bacteria. The hexane extract showed no lethality against brine shrimp while the extract exhibited negative insecticidal activity. The Hexane extract exhibited non-significant activity against *Salmonela typhi* with zone of inhibition of 10mm. non-significant leishmanicidal activity with IC_{50} value of >100 µgml^{-1}.

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